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(54) OXYGEN-ABSORBING AGENTS

(71) We, MERCK PATENT GESELLSCHAFT MIT BESCHRANKTER HAFTUNG, of 250, Frankfurter Strasse, 61 Darmstadt, Federal Republic of Germany, a Joint-Stock Company organised under the laws of the Federal Republic of Germany, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:-

The present invention is concerned with oxygen-absorbing agents and with a process for the production of an oxygen-poor or oxygen-free atmosphere for culturing anaerobic bacteria with the use of these oxygen-absorbing agents.

Oxygen-absorbing agents have long been used for the reduction or removal of atmospheric oxygen for anaerobic culture processes. Thus, for example, use has been made of mixtures of pyrogallol powder, sodium carbonate and kieselguhr, which are thoroughly moistened either by adding water or by water vapour from the nutrient substrate and then absorb oxygen. J. Clin. Microbiol., 1975, page 527 also described a mixture of steel wool and acidified copper sulphate solution for this purpose.

The known mixtures have the disadvantage that their absorption ability for oxygen is not optimal. In the case of the abovementioned mixture described in J. Clin. Microbiol., an acidic copper sulphate solution must first be prepared with which the steel wool is then saturated. After some time, the excess of this solution must be discarded.

It is an object of the present invention to provide an oxygen-absorbing agent which possesses an optimum absorption ability, is simple to handle and can be used immediately.

Thus, according to the present invention, there is provided an oxygen-absorbing agent, comprising an adsorption agent, metal powder or turnings and an activator.

The present invention also provides a process for the production of an oxygen-poor or oxygen-free atmosphere for culturing anaerobic bacteria, wherein an oxygen-absorbing agent according to the present invention is brought into contact with a reaction mediator.

Surprisingly, we have found that the agent according to the present invention, in comparison with the mixture described in J. Clin. Microbiol., possesses a 5 to 10 times better oxygen absorption ability, referred to the amount of iron.

The adsorption agent contained in the agent according to the present invention serves to suck up the liquid reaction mediator and thus to prevent a deliquescence of the mixture. Examples of adsorption agents which can be used include kieselguhr, silica gel and cellulose, kieselguhr being preferred.

Examples of metal powders or turnings which can be used include those of heavy metals, such as iron, manganese, cobalt, nickel and the like, iron powder being preferred.

Examples of activators which can be used include organic acids, such as citric acid, tartaric acid, dilute acetic acid and the like, dilute mineral acids, such as hydrochloric acid and sulphuric acid, as well as complexing agents and salts of noble metals. A preferred activator according to the present invention is citric acid.

In many cases, it is desirable to add sodium carbonate to the oxygen-absorbing agent in order, for example, to provide in the closed culture vessel a certain pressure equilisation for the absorbed oxygen or because some bacteria require an increased content of carbon dioxide in the atmosphere for optimum growth.

The weight ratio of the individual mixture

components adsorption agent : metal powder : activator : sodium carbonate is preferably about 8:4:3:1. In the case of mixtures without carbon dioxide evolution, i.e. without the addition of sodium carbonate, this weight ratio is preferably from about 8:4:2 to 8:4:1. The ratio of sodium carbonate to citric acid activator depends essentially upon the desired amount of carbon dioxide. The ratio of adsorption agent to metal powder can be varied within wide limits. In the case of kieselguhr and iron powder, the weight ratio can be from 5:1 to 1:1 and is preferably about 2:1. When substantially more or less iron is employed, the oxygen-absorption ability of the agent according to the present invention decreases.

For the production of an oxygen-poor or oxygen-free atmosphere, such as is necessary, for example, for culturing anaerobic bacteria, the dry mixture according to the present invention is brought into contact with a reaction mediator. The reaction mediator can be a solvent, for example water or ethylene glycol or a liquid acid, for example acetic acid, dilute hydrochloric acid, sulphuric acid or the like, which, in turn, simultaneously act as activator and reaction mediator. The preferred reaction mediator is water.

As soon as the reaction mediator comes into contact with the oxygen-absorbing agent, the moist mixture begins to absorb the oxygen comparatively quickly, with the simultaneous evolution of carbon dioxide when sodium carbonate is present.

The following Examples are given for the purpose of illustrating the present invention:-

Example 1

For culturing anaerobic bacteria, 30 g. of a mixture consisting of

5.0 kg. kieselguhr,
2.5 kg. iron powder,
1.9 kg. citric acid and
0.6 kg. sodium carbonate

are filled into a paper sachet and placed in an anaerobic vessel next to Petri dishes containing anaerobic cultures. 20 ml. water are introduced with a syringe into the paper sachet and the vessel is closed and left to incubate. After only a few minutes, a practically oxygen-free atmosphere is produced in the anaerobic vessel.

Example 2

Analogously to Example 1, aliquot parts of a mixture consisting of

5.0 kg. kieselguhr,
2.5 kg. cobalt powder,
1.5 kg. tartaric acid and
0.5 kg. sodium carbonate

are used as oxygen-absorbing agent for culturing anaerobic bacteria.

Example 3

For the production of an oxygen-poor atmosphere without the simultaneous evolution of carbon dioxide, analogously to Example 1, there are used aliquot parts of one of the following mixtures:

a) 5.0 kg. silica gel,
2.5 kg. iron powder and
1.0 kg. citric acid

b) 5.0 kg. kieselguhr,
2.5 kg. cobalt powder and
0.8 kg. tartaric acid.

WHAT WE CLAIM IS:-

1. An oxygen-absorbing agent, comprising an adsorption agent, metal powder or turnings and an activator.

2. An oxygen-absorbing agent according to claim 1, comprising kieselguhr, iron powder and citric acid.

3. An oxygen-absorbing agent according to claim 1 or 2, which additionally contains sodium carbonate.

4. An oxygen-absorbing agent according to claim 1, substantially as hereinbefore described and exemplified.

5. A process for the production of an oxygen-poor or oxygen-free atmosphere for culturing anaerobic bacteria, wherein an oxygen-absorbing agent according to any of claims 1 to 4 is brought into contact with a reaction mediator.

6. A process according to claim 5, wherein the reaction mediator is a solvent.

7. A process according to claim 6, wherein the solvent is water.

8. A process according to claim 5 for the production of an oxygen-poor or oxygen-free atmosphere, substantially as hereinbefore described and exemplified.

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